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Comparison of the pathogenesis of acute equine herpesvirus 1 (EHV-1) infection in the horse and the mouse model: a review

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Abstract

The mouse models of the respiratory and abortion forms of equine herpesvirus 1 (EHV-1) infection have been used to investigate the vaccine potential of various EHV-1 immunogens, the effect of antiviral agents on EHV-1 infection and the pathogenicity of EHV-1 strain variants and deletion or insertional mutants. This review examines the similarities and differences in the pathogenesis of primary EHV-1 infection in the natural host, the horse, and in the mouse by comparing tissue tropism, clinical signs of infection, the effects of EHV-1 on pregnancy, haematological changes following infection, viral clearance, histopathology and latency. The evidence suggests that the mouse model provides a valid method for investigation of virological and histopathological aspects of EHV-1-induced disease in the horse. However, the extent to which useful and valid comparisons and extrapolations can be made of immunological parameters from mouse to horse is yet to be determined. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Equine herpesvirus-1 (EHV-1) is a major cause of respiratory disease, abortion, perinatal mortality, and occasionally neurological signs in horses (Allen and Bryans, 1986; Crabb and Studdert, 1995). The virus is endemic worldwide and infection has a high morbidity and is easily spread by inhalation of saliva or nasal discharge, fomites or contaminated feed or water (Allen and Bryans, 1986). A vaccine which prevents EHV-1-

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induced abortion has yet to be produced (Gilkerson et al., 1997a). Apart from the inherent difficulties associated with immunising against any herpesvirus infection, experimental work with horses is expensive and labour-intensive. Consequently, an animal model has been sought with which to evaluate experimental vaccines, in vivo properties of the virus and antiviral drugs.

Early models, which used intracerebral inoculation of suckling mice (Patel and Edington, 1983; Nowotny et al., 1987) and intranasal or intraperitoneal inoculation of the hamster (Wilks and Coggins, 1977; Stokes et al., 1989), had inherent difficulties, including the inappropriate modes of infection and the types of disease produced. Subsequently, a murine model of EHV-1-induced respiratory disease, which mimicked many of the features of EHV-1 infection in the natural host, was developed using mice infected intranasally under general anaesthesia (Awan et al., 1990). Considerable effort has gone into identifying susceptible strains of mice. Most commonly, BALB/c mice have been used, as other strains of mice were found less receptive to infection (Awan et al., 1990; Walker et al., 1998b). However, Alber et al. (1995) found that EHV-1 infection in C3H (H-2^k) mice engendered more pronounced immune responses than in BALB/c mice. During the course of these studies, mice infected late in pregnancy were shown to abort or give birth to moribund young, giving rise to the abortion model of EHV-1 infection (Awan et al., 1991).

The development of a useful model has lead to a considerable number of studies investigating the pathogenesis and immune responses to EHV-1 infection in the mouse (Field et al., 1992; Azmi and Field, 1993a, b; Inazu et al., 1993; Alber et al., 1995; Awan et al., 1995; Baxi et al., 1995; Csellner et al., 1995; Marshall and Field, 1997; Bartels et al., 1998; Smith et al., 1998b; Walker et al., 1998a, b). As well, the model has been used to investigate the vaccine potential of various EHV-1 immunogens (Tewari et al., 1994, 1995; Osterrieder et al., 1995; Stokes et al., 1996, 1997; Kukreja et al., 1998a, b; Packiarajah et al., 1998; Ruitenberg et al., 1998), the effect of antiviral agents on EHV-1 infection (Field and Awan, 1990; Gibson et al., 1992b; Awan and Field, 1993) and the pathogenicity of EHV-1 strain variants (Van Woensel et al., 1995; Colle et al., 1996) and deletion or insertional mutants (Slater et al., 1993; Osterrieder et al., 1996; Fitzmaurice et al., 1997; Marshall et al., 1997; Neubauer et al., 1997; Csellner et al., 1998; Walker et al., 1998b). This review seeks to elucidate the similarities and differences in the pathogenesis of primary EHV-1 infection in the natural host the horse and in the mouse.

2. Tissue tropism

Following experimental or naturally-occurring infection of horses, infectious EHV-1 has been isolated from nasal epithelium, nasal turbinates, pharynx, trachea, bronchi, bronchioles, lung, cerebrum, thyroid, uterus, conjunctiva, submandibular and inguinal lymph nodes, kidney, the endothelium of epididymis and testis, semen and the pregnant uterus (Edington et al., 1986; Whitwell and Blunden, 1992; Smith et al., 1993; Kydd et al., 1994; Tearle et al., 1996). Virus has also been isolated from the lung, liver, spleen, thymus and adrenals of foals whose mares were infected (Bryans et al., 1977; Dixon et al., 1978; Whitwell and Blunden, 1992). Immunoperoxidase and immunofluorescent

staining have revealed virus in endothelial cells of endometrium, placenta and the umbilical vein and major foetal vessels (Edington et al., 1986; Smith et al., 1996).

In the EHV-1-infected mouse, infectious virus can be isolated readily from nasal turbinates, trachea, lungs, olfactory bulbs, brain and eye, rarely from liver but not from spleen, cervical lymph nodes, adrenals, heart, kidney or pancreas (Awan et al., 1990; Inazu et al., 1993; Csellner et al., 1995; Baxi et al., 1996; Marshall and Field, 1997). In addition, nested PCR has demonstrated viral DNA in spleen and trigeminal ganglia (Baxi et al., 1996; Bartels et al., 1998). In infected pregnant mice, virus can be isolated from foetus, placenta and uterus (Awan et al., 1991; Kukreja et al., 1998b; Walker et al., 1998b), and in situ hybridization has demonstrated virus in chorionic epithelium and endothelium and placental trophoblasts (Awan et al., 1995).

Following experimental EHV-1 infection, not all aborted equine foetuses have been virologically positive or showed characteristic herpesvirus-specific histopathological lesions, despite there being no other demonstrable reason for abortion (Smith et al., 1992). There may be vascular abnormalities in a placenta negative for virus, which suggested that in EHV-1 infection late in pregnancy, abortion may be due to maternal factors alone, without infection of the foetus. Similarly in the mouse, viral infection of the foetus and/or placenta may be absent, yet vascular lesions of the placenta occur quite commonly (Walker et al., 1998b). As well, a placenta may be positive for virus, yet its foetus may be negative, and vice versa (Kukreja et al., 1998b). Within a single litter from an infected dam, some foetuses may be affected, yet others will be normal (Walker, unpublished data). However, in a case of naturally-occurring EHV-1-induced abortion of twin foals, both foetuses were infected with virus (Dunn et al., 1993).

It should be pointed out that despite many of the similarities in tissue tropism as outlined above, there is no documented evidence of transmission or spread of EHV-1 among mice (Csellner et al., 1995). Therefore, the model does not necessarily offer useful insight into the epidemiology or cycle of EHV-1 in the natural host.

3. Clinical signs of infection

Primary EHV-1 infection in the horse may occur before weaning, with foals as young as 30 days showing serological evidence or EHV-1 infection (Gilkerson et al., 1997b). Spread amongst susceptible foals then occurs and is amplified when foals are mixed at weaning (Gilkerson et al., 1997b). Classically, the incubation period is described as being between 1 and 2 days, but sometimes extending to 10 days post-infection (pi) (Dutta et al., 1980; Allen and Bryans, 1986; Ostlund et al., 1991). In experimentally-infected horses, respiratory signs peak at days 4 to 5 pi and are characterised by rhinopharyngitis, which is initially serous but later becomes mucopurulent as the infection proceeds, as well as tracheobronchitis, rhinopneumonitis, lymphadenomegaly, depression and inappetence (Allen and Bryans, 1986; McCulloch et al., 1993). Resolution of such signs usually occurs within 12 days pi (Gibson et al., 1992a). Clinical signs in naturally-infected foals have not been described with any certainty and it is possible that the disease follows a benign course with rapid resolution and minimal morbidity. Neurological signs

have been described principally in adult horses, occur rarely and include ataxia, paresis or paralysis. These are the consequences of both direct EHV-1 infection and vascular changes in central nervous tissues as well (Jackson et al., 1977; Allen and Bryans, 1986). Experimentally-infected horses exhibit a febrile response, which begins with the onset of clinical signs, peaking at day 2 pi and lasting for up to 7 days (Chong and Duffus, 1992). It may be diphasic, peaking a second time at day 6 pi (Gibson et al., 1992c; Kydd et al., 1994), and Allen and Bryans (1986) observed that this second peak tended to coincide with the onset of viraemia.

Intranasal infection of EHV-1 in the mouse also causes respiratory signs – polypnoea and dyspnoea, as well as signs of systemic illness. The mice become quiet, are dehydrated, have ruffled fur, hunched posture, and occasionally mucopurulent conjunctivitis, evident within 1 day pi and resolved by day 6 to 12 pi (Awan et al., 1990; Inazu et al., 1993; Walker et al., 1998a). Neurological signs are not usually observed, although hindlimb paresis has been reported (Awan et al., 1990). Mice lose weight from 12 h pi, and preinoculation body weights are not regained until at least 14 days pi (Awan et al., 1990; Colle et al., 1996; Marshall et al., 1997; Walker et al., 1998a). The onset and severity of clinical signs in the EHV-1-infected mouse vary with the dose (Azmi and Field, 1993a; Smith et al., 1998a; Walker, unpublished data) and strain of inoculating virus (Van Woensel et al., 1995). For example, the more pathogenic strain, Ab4, originally isolated from a horse with neurological signs, may kill mice (Awan et al., 1990; Field et al., 1992; Azmi and Field, 1993b; Baxi et al., 1996), whereas the KyA strain causes mild disease only (Colle et al., 1996). EHV-1 infection in the mouse is accompanied by a marked fall in rectal temperature (<37.5°C), which is lowest between days 1 and 2 pi (Walker et al., 1998a).

4. Effects of EHV-1 on pregnancy

EHV-1 infection in pregnant mares may result in abortion, depending to some extent on the stage of gestation. EHV-1-induced abortion usually occurs during the last 4 months of gestation and Doll and Bryans (1963) were of the opinion that there may be some resistance to abortion if infection occurs early in pregnancy. This was thought to be related to the reduced number of vascular lesions and viral antigen expression in the endothelial cells of early pregnant mares compared with mares infected late in pregnancy (Smith et al., 1996). Abortion usually occurs rapidly and with little warning. If the foetus is infected with EHV-1 late in pregnancy, it may be born dead, or alive, but weak, depressed, polypnoeic and febrile and die within hours or days (Hartley and Dixon, 1979). Other foals may be healthy at birth but succumb to the effects of EHV-1-induced tissue damage within the first week of life (Bryans et al., 1977).

The effects of EHV-1 on the outcome of murine pregnancy also depends on the stage of pregnancy at which the dams are infected. Infection in early gestation is associated with resorption of foetuses (Awan et al., 1995). At mid- and late gestation, foetuses may be born prematurely, either dead or moribund and die soon after. Infection with EHV-1 relatively late in gestation may not necessarily affect gestational length (Kukreja et al., 1998b), just as there is no indication that perinatal EHV-1 infection in horses

reduces gestational age of the foetus (Dixon et al., 1978). However, foetal size in utero may be reduced compared with uninfected controls (Walker et al., 1998b). EHV-1 infection in the pregnant horse does not invariably cause abortion (Smith et al., 1992), and similarly, some mouse litters are unaffected by infection (Awan et al., 1991; Walker et al., 1998b).

5. Haematological changes following infection

Experimentally-infected horses develop a leucopaenia, due to both a neutropaenia and a T cell lymphopaenia, during acute EHV-1 infection and a B cell lymphocytosis during the convalescent period (Bumgardner et al., 1982; Scott et al., 1983; Allen and Bryans, 1986; Gibson et al., 1992a; McCulloch et al., 1993). McCulloch et al. (1993) postulated that the lymphocytopaenia may be due to T cells becoming entrapped in lymph nodes draining the infection site or perhaps to selective infection and consequent destruction of T cells by EHV-1. Neither of these events appear to occur following EHV-1 infection in the mouse, where a leucocytosis, due to a neutrophilia and a B cell lymphocytosis, during the first few days of infection has been noted (Csellner et al., 1998; Packiarajah et al., 1998; Walker et al., 1998a). These differences may reflect the species variation in their response to disease or the dissimilarity between the leucocyte responses of outbred horses and inbred SPF mice.

6. Viral clearance

The period of virus shedding from the nasopharynx of experimentally-infected horses has been reported as varying between 5 and 14 days pi, with most clearing by day 12 pi (Bryans, 1969; Allen and Bryans, 1986; Gibson et al., 1992c; Hannant et al., 1993; Tewari et al., 1993), although virus isolation may extend beyond clinical recovery (Chong and Duffus, 1992). Similarly, virus clearance from mouse lungs following primary EHV-1 infection has been reported variously as occurring from 5 to 12 days pi, again depending on the dose administered and infective viral strain (Awan et al., 1990; Azmi and Field, 1993b; Inazu et al., 1993; Slater et al., 1993; Tewari et al., 1994; Alber et al., 1995; Csellner et al., 1995; Walker et al., 1998a).

Studies on experimental infection in SPF foals found viraemia, detected using buffy coat preparations and infectious centre assays, between days 3 to 5 pi and 12 to 16 pi (Gibson et al., 1992d). In individual pregnant mares following experimental infection, the onset of viraemia varied from days 3 to 14 pi, and for the duration of one study, virus was recovered continuously from leucocytes from the time it first appeared (Gleeson and Coggins, 1980; Smith et al., 1996). In mice, the detection of viraemia using infectious centre assays of buffy coat preparations has been variable, reported as occurring from days 1 to 5 i.e. during the acute phase of infection by some workers (Awan et al., 1990; Field and Awan, 1990; Field et al., 1992; Gibson et al., 1992b; Azmi and Field, 1993b) yet undetectable by others (Kukreja et al., 1998a; Baxi et al., 1996). However, viraemia was still discernible at 8 days pi using a second round of nested PCR (Baxi et al., 1996).

Van Woensel et al. (1995) were unable to detect virus in serum, suggesting that the viraemia is cell-associated.

7. Histopathology

Following experimental EHV-1 infection in the horse, there is inflammation, necrosis and intranuclear inclusion bodies in the nasal, pharangeal and occasionally tracheal epithelium, and the germinal centres of the mandibular, pharyngeal and bronchial lymph nodes (Allen and Bryans, 1986; Edington et al., 1986; Kydd et al., 1994). These studies showed initially a patchy, acute bronchiolitis, interstitial oedema and neutrophilic infiltration of the terminal bronchioles. As infection progressed, peribronchiolar and perivascular aggregations of mononuclear cells appeared, with the pulmonary interstitial changes peaking at day 9 pi (Allen and Bryans, 1986; Kydd et al., 1994). Many of the lesions were vascular, with thrombi in the vessels of the nasal mucosa and pulmonary vasculitis (Jackson et al., 1977; Edington et al., 1986; Whitwell and Blunden, 1992; Kydd et al., 1994). A necrotising vasculitis has also been reported in the testis and epididymis of experimentally-infected colts (Tearle et al., 1996) as well as vasculitis in the endometrium, regardless of the pregnancy status of the mare (Jackson et al., 1977). In both natural and experimentally-induced EHV-1 infections associated with neurological disease, thrombi can be found in vessels of the grey and white matter of the CNS and the leptomeninges, followed by focal vasculitis and necrosis of the brain stem, cerebrum and white matter of the spinal cord (Jackson et al., 1977; Edington et al., 1986; Whitwell and Blunden, 1992).

The lung histopathology in EHV-1-infected mice is similar to that of the horse and is characterised by an acute focal alveolitis and bronchiolitis, eosinophilic intranuclear inclusion bodies in bronchiolar epithelial cells, focal necrosis of pneumocytes and perivascular and peribronchiolar aggregates of mixed inflammatory cells, initially neutrophilic but tending towards mononuclear as the infection proceeds (Awan et al., 1990; Field and Awan, 1990; Csellner et al., 1995; Van Woensel et al., 1995; Bartels et al., 1998; Walker et al., 1998a). Resolution of specific herpesvirus lesions resulted in absence of intranuclear inclusion bodies and parenchymal necrosis by day 4 pi and the cellular response was reduced by 7 days pi (Walker et al., 1998a). No inflammatory or degenerative processes or vascular lesions have been observed in the CNS of mice (Bartels et al., 1998).

As a consequence of experimental infection with EHV-1 in pregnant mares, the endometrium has been described as congested, with severe and widespread vascular changes including perivascular oedema, extensive areas of ischaemia associated with avascular necrosis and perivascular infiltration of lymphocytes, neutrophils and monocytes (Smith et al., 1992; Carlton and McGavin, 1995). Unlike in the mare, histological abnormalities have not been observed in uterus of EHV-1-infected pregnant mice. However, in the placenta, congestion of the middle layer of the trophoblast and a reduction in normal trophoblastic tissue has been observed commonly from days 2 to 4 pi (Awan and Field, 1993; Awan et al., 1995; Walker et al., 1998b) and necrosis of the middle layer of the trophoblast, presumably following congestion and consequent

ischaemia, was seen at day 3 pi (Walker et al., 1998b). Similarly, in the equine placenta, patchy congestion and necrosis of the chorion and petechial haemorrhages in the stroma have been reported (Smith et al., 1992, 1993).

Histological differences following natural EHV-1 infection have been observed between aborted foetuses and those which died perinatally. Hartley and Dixon (1979) described the most consistent features in the aborted foetus as necrotising bronchiolitis, interstitial pneumonitis, focal hepatic necrosis and necrosis of the germinal centres in all lymphocytic tissues. Eosinophilic inclusion bodies were found commonly in the nuclei of the bronchiolar and alveolar epithelium as well as occasionally in the hepatic parenchymal cells (Dixon et al., 1978). In perinatal EHV-1 infection, where the foals might survive for some hours postnatally, focal hepatic necrosis was less frequently observed but there was massive atelectasis with interstitial pneumonia, splenic and thymic hypoplasia and adrenal hyperplasia with focal necrosis and haemorrhage (Bryans et al., 1977; Hartley and Dixon, 1979). In mouse pups born alive but which died perinatally, the lungs showed consolidation and cytopathic changes typical of EHV-1 infection in the bronchiolar epithelium (Awan et al., 1991, 1995; Awan and Field, 1993). However, no histological abnormalities were seen in any foetus collected at days 1–5 pi from EHV-1-infected dams before parturition had occurred, which may reflect variation in the inoculating strain of virus (Walker et al., 1998b).

8. Latency

In common with other herpesviruses, EHV-1 can establish a latent infection, which can become reactivated after the primary infection has resolved (Allen and Bryans, 1986). Experimental reactivation by administration of immunosuppresive agents has resulted in shedding EHV-1 into nasal mucus and in some cases, viraemia has been induced (Edington et al., 1985; Gibson et al., 1992a). In healthy horses previously infected with EHV-1 but not currently shedding virus, co-cultivation of explanted trigeminal ganglia yielded infectious virus (Slater et al., 1994) and viral DNA has been detected by PCR in trigeminal ganglia, olfactory nerve, spleen, lymphoid tissue associated with the respiratory tract and peripheral blood leucocytes (PBL) (Welch et al., 1992; Edington et al., 1994; Slater et al., 1994). Latency-associated transcripts (LATs) have been detected using in situ hybridisation in low numbers of neurones of the trigeminal ganglia, which demonstrated that some of the cells which contain EHV-1 transcribe regions of the viral genome during latency (Baxi et al., 1995).

Virus can also be reactivated following various stimuli in mice previously infected with EHV-1, with infectious virus isolated from nasal turbinates and PBL (Field et al., 1992). Consistent with this, viral DNA was detected by PCR in trigeminal ganglia (Baxi et al., 1996). Also, low levels of virus DNA could still be detected in trigeminal ganglia, olfactory bulbs and PBL in mice in which infectious virus was no longer detectable (Baxi et al., 1996). In that series of experiments, LATs could not be detected using in situ hybridisation, which may indicate either that they were not present or were below the level of detection. However, using in situ PCR and expression of a *lacZ* reporter gene,

Marshall and Field (1997) have shown that the mitral/tufted neurons within olfactory bulbs were probable sites of EHV-1 latency in the mouse.

9. Conclusion

Laboratory animals may be convenient and valuable tools for use in experimental systems of disease in large animals. However, they are often criticised because of their failure to provide relevance to that disease under investigation in the target species. The comparative analysis in this brief review suggests that the mouse model provides a valid method for investigation into virological and histopathological aspects of EHV-1-induced disease in the horse. The extent to which useful and valid comparisons and extrapolations can be made of immunological parameters from mouse to horse is yet to be determined. Nevertheless, although the murine respiratory model of EHV-1 infection is not perfect, its extensive use has given direction for vaccine strategies, while the abortion model may yet prove of some use in investigation of aspects of EHV-1-induced abortion.

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